Spectrophotometric Method for Simultaneous Estimation of Lopinavir and Ritonavir in bulk and tablet dosage form

Jaiprakash N. Sangshetti\(^1\)*, Sachin Bhojane\(^1\), Baig Salim Rashid\(^1\), Indrajeet Gonjari\(^2\)

\(^1\) Y. B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Rauza Baugh, Aurangabad-431001
\(^2\) Government College of Pharmacy, Karad-415110 (M.S.), India.

\(^*\) Corres. Author: jnsangshetti@rediffmail.com

Abstract: Simple area under curve method for determination of Lopinavir and Ritonavir in bulk and tablet formulation was developed and validated as per ICH guidelines. The \(\lambda_{\text{max}}\) of lopinavir and ritonavir were found to be 240nm and 260nm respectively. The linearity range was found 10-60 \(\mu\)g/mL for lopinavir and 5-30 \(\mu\)g/mL for ritonavir. In the tablets dosage form lopinavir and ritonavir were estimated as 99.82% and 99.89% respectively. The lower limit of detection (LOD) was found to be 1.1 \(\mu\)g/mL for lopinavir and 1.414 \(\mu\)g/mL for ritonavir and the limit of quantization (LOQ) was found to be 3.33 \(\mu\)g/mL and 4.2 \(\mu\)g/mL respectively for lopinavir and ritonavir. The validated spectrophotometric method employed proved to be simple, economical, precise and accurate.

Keywords: Lopinavir, Ritonavir, UV Spectroscopy, AUC Method.

1. Introduction

The mechanism of action of lopinavir and ritonavir is to inhibit the HIV viral Protease enzyme. This prevents cleavage of the gag-pol polyprotein and, therefore, improper viral assembly result. This subsequently results in non-infective, immature viral particles. The chemical name for lopinavir is (2S)-N-[(2S, 4S, 5S)-5-{2-(2, 6-dimethylphenoxy) acetamido]- 4-hydroxy-1, 6-diphenylhexan -2-yl]-3- methyl-2-(2-oxo-1,3-diazinan-1-yl)butanamide (1,7). The chemical structure for lopinavir is shown in figure 1(A)\(^(7)\). The chemical name for ritonavir is 1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-{{[methyl([2-(propan-2-yl)-1,3-thiazol-4-yl)methyl}]carbamoyl]amino} butanamido]-1,6-diphenylhexan-2-yl\] carbamate. It has the structural formula and shown in the figure 1(B)\(^(9)\).

There are some methods reported for estimation of lopinavir and ritonavir by spectroscopy\(^1, 4\) HPLC and HPTLC. But no other method such as area under curve spectrophotometric method was reported for the

http://www.sphinxsai.com/framesphinxsaichemtech.htm
quantitative determination of lopinavir and ritonavir in bulk and pharmaceutical dosage forms. The developed method was simple, precise, specific, and accurate and validated as per ICH guidelines \(^{(2)}\). The statistical analysis proved that method is reproducible and selective for the analysis of lopinavir and ritonavir in bulk drug and tablet formulations.

![Chemical structure of Lopinavir](image1.png)  
![Chemical structure of Ritonavir](image2.png)  

**Figure 1.** Chemical structure of Lopinavir and Ritonavir

2. EXPERIMENTAL

2.1 Material

For the development process, we used UV-Visible double beam spectrophotometer (Jasco, Model UV-V-630). Methanol GR grade (Dodal Enterprises, Aurangabad) and water double distilled water. Pure lopinavir and ritonavir were obtained as gift sample and the drug was used as such for further analysis. Formulations were purchased from the local pharmacies and used for analysis.

2.2 Preparation of working standard drug solution

A stock solution of lopinavir and ritonavir (100µg/ml) was prepared, by accurately weighing 10 mg of each drug and dissolving in separate 100 ml volumetric flasks. They were dissolved first in 50 ml of Methanol and then the volume was making up to the mark to get 100µg/ml. For each drug, appropriate liquid were pipette out from the standard stock solution into a series of 10mL volumetric flasks, to get a set of dilutions for each drug.

2.3 Preparation of Sample Solution

The average tablet mass was calculated from the mass of 20 tablets of Lopimune (200 mg lopinavir and 50 mg ritonavir) tablet. They were then finely ground, homogenized and 40mg lopinavir and 10mg ritonavir equivalent portion of the powder was weighed accurately, transferred into a 100 ml of volumetric flask and diluted to scale with methanol. The mixture was sonicated for at least 20 min to aid dissolution and then filtered through a Whatman No 42 paper. Approximate dilutions were made at concentrations of 40µg/ml and 10µg/ml in methanol.

2.4 Selection of Analytical wavelength

By appropriate dilutions with methanol 40µg/ml solution of lopinavir and 10 µg/ml solution of ritonavir were prepared separately. These were scanned in the spectrum mode from 400nm to 200nm. As shown in figure 2 the wavelength range for lopinavir was chosen between 255-264 nm and for ritonavir it was chosen between 230 – 245 nm.

2.5 Procedure for analysis of tablet formulation

Twenty tablets of lopinavir and ritonavir in combination were weighed; their average weight was determined and finally crushed to powder sample. From the triturate, tablet powder equivalent to 40mg of lopinavir and
10mg of ritonavir was weighed and transferred to 100ml volumetric flask and dissolved in 50ml methanol and Finally the volume was made upto the mark with methanol. The solution is subjected to ultrasonification for 30min and then filtered through Whatman filter paper No.41. This tablet solution was further diluted to obtain 40µg/ml of lopinavir and 10µg/ml of ritonavir respectively. The mixed sample solutions were analyzed to obtain spectra and the AUC is recorded using wavelength range from 255 – 264nm for lopinavir and 230 – 245nm for ritonavir were noted.

2.6 Calibration curve

Method: Area under Curve Method

For the selection of analytical wavelength, 40 µg/mL solution of lopinavir and 10 µg/mL solution of ritonavir was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. From the spectra of drugs λ max of lopinavir and ritonavir, was selected 260 nm and 240 nm respectively for the analysis. The calibration curve was prepared in the concentration range of 10-60µg/mL at 259nm for lopinavir and 5-30µg/mL at 240nm for ritonavir. By using the calibration curve, different concentrations of the sample solution were calculated.

![Figure 2. Overlaid Spectra Showing Area under Curve of lopinavir and ritonavir](image

Equation used for determination of concentrations of LOPI and RITO:

\[
X_{\text{RITO}}^{255-640} \times \text{AUC}_{230-245} = X_{\text{RITO}}^{230-245} \times \text{AUC}_{255-264}
\]

\[
C_{\text{LOPI}} = \frac{X_{\text{RITO}}^{255-264} \times X_{\text{LOPI}}^{230-245} - X_{\text{RITO}}^{230-245} \times X_{\text{LOPI}}^{255-264}}{X_{\text{RITO}}^{255-264} \times X_{\text{LOPI}}^{230-245} - X_{\text{RITO}}^{230-245} \times X_{\text{LOPI}}^{255-264}}
\]

\[
X_{\text{LOPI}}^{230-245} \times \text{AUC}_{255-264} = X_{\text{LOPI}}^{255-264} \times \text{AUC}_{230-245}
\]

\[
C_{\text{RITO}} = \frac{X_{\text{RITO}}^{255-264} \times X_{\text{LOPI}}^{230-245} - X_{\text{RITO}}^{230-245} \times X_{\text{LOPI}}^{255-264}}{X_{\text{RITO}}^{255-264} \times X_{\text{LOPI}}^{230-245} - X_{\text{RITO}}^{230-245} \times X_{\text{LOPI}}^{255-264}}
\]

Where AUC_{230-245} and AUC_{255-264} are the area under curves of solution at wavelength range between 230 – 245 nm (ritonavir) and 255 – 264 nm (lopinavir) respectively.
3. RESULT AND DISCUSSION

Calibration standards for lopinavir covering the range of 10-60 µg/mL and for ritonavir the range of 5-30 µg/mL were prepared by the method mentioned above and the serial dilutions were made with methanol. The spectrum was presented in Figure 2. The calibration curve was obtained by plotting the intensity by absorbance of the lopinavir and ritonavir versus respective analyte concentration. Regression analysis of the calibration curve showed a linear relationship between the intensity of absorbance of lopinavir and ritonavir respectively. The wavelength range for lopinavir was chosen between 255-264 nm and for ritonavir it was chosen between 230 – 245 nm. The various validation parameters are presented in Table 1.

Table 1. Summary of Validation Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Data for lopinavir</th>
<th>Data for ritonavir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>10-60 µg/ml</td>
<td>5-30 µg/ml</td>
</tr>
<tr>
<td>Regression equation</td>
<td>( y = 0.003x + 0.001 )</td>
<td>( y = 0.014x + 0.006 )</td>
</tr>
<tr>
<td>Correlations coefficient</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Limit of Detection (µg/mL)</td>
<td>1.1</td>
<td>1.414</td>
</tr>
<tr>
<td>Limit of Quantitation (µg/mL)</td>
<td>3.33</td>
<td>4.2</td>
</tr>
<tr>
<td>Accuracy</td>
<td>99.82%</td>
<td>99.89%</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific (Spectra match with standard drug)</td>
<td>Specific (Spectra match with standard drug)</td>
</tr>
<tr>
<td>Robustness</td>
<td>Robust</td>
<td>Robust</td>
</tr>
<tr>
<td>Ruggedness</td>
<td>%RSD Less than 1%</td>
<td>%RSD Less than 1%</td>
</tr>
<tr>
<td>Precision (RSD, %)</td>
<td>1.98</td>
<td>1.98</td>
</tr>
<tr>
<td>Intraday (n=3)</td>
<td>1.65</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>1.95</td>
<td>0.93</td>
</tr>
<tr>
<td>Interday (n=3)</td>
<td>1.997</td>
<td>1.88</td>
</tr>
<tr>
<td></td>
<td>1.992</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>1.86</td>
<td>1.69</td>
</tr>
</tbody>
</table>

Table 2. Analysis of Tablet Formulation

<table>
<thead>
<tr>
<th></th>
<th>Lopinavir Level of % Recovery (( \lambda_{max} =260 ) nm)</th>
<th>Ritonavir Level of % Recovery (( \lambda_{max} =240 ) nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount Present (mg)</td>
<td>80 100 120</td>
<td>80 100 120</td>
</tr>
<tr>
<td>Amount of Standard Added (mg)</td>
<td>200 200 200</td>
<td>50 50 50</td>
</tr>
<tr>
<td>Total Amount Recovered(mg)</td>
<td>359.79 399.97 437.90</td>
<td>89.99 99.80 109.90</td>
</tr>
<tr>
<td>% Mean</td>
<td>99.82</td>
<td>99.89</td>
</tr>
<tr>
<td>SD</td>
<td>0.252</td>
<td>0.0901</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.216</td>
<td>0.092</td>
</tr>
</tbody>
</table>

4. CONCLUSION

Simple area under curve method for determination of lopinavir and ritonavir in bulk and tablet formulation was developed and validated as per ICH guidelines. The validated spectrophotometric method employed proved to be simple, economical, precise and accurate. Thus it can be used as IPQC test and for routine simultaneous determination of lopinavir and ritonavir in tablet dosage form.
5. REFERENCES


*****